THE ANTIFUNGAL COATING OF DIFFERENT WALL FINISHING TOWARDS GROWTH INHIBITION OF *ASPERGILLUS NIGER*

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DEDICATION

Specially dedicated to my beloved mother, Hajah Sa'diah binti Misman
and to my late father Haji Parjo bin Singo, brothers and sisters,
my supervisor Assoc. Prof. Dr. Norshuhaila binti Mohamed Sunar
and co-supervisor Assoc. Prof. Dr. Abdul Mutalib bin Leman
my husband Akmal Hakim bin Remle
and to all my friends especially, Nurulliza binti Mohamad,Yusma Yusnida binti
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ABSTRACT

Indoor air quality (IAQ) is an essential factor which directly affects human and the comfort of building occupants. The concentration of fungi, humans, animals and others contribute to indoor air pollutants and can cause a syndrome known as sick building syndrome. In this study, the growth of fungi in a lecturers' room at Universiti Tun Hussein Onn Malaysia (UTHM) was investigated with the application of three types of antifungals namely potassium sorbate, calcium benzoate and zinc salicylate. The study was conducted to enumerate and evaluate the growth of *Aspergillus niger* (*A. niger*). Four different types of wall finishings were utilized; namely acrylic paint, glycerol-based paint, thin wallpaper and thick wallpaper. These wall finishings were applied after being incorporated with antifungals on three different building materials; namely plasterboard, concrete and wood. Each sample was placed with 50µl of the spore suspension on a petri-dish containing malt extract agar. The sample was incubated at the relative humidity and temperature of 90% and 32ºC respectively, based on the results of preliminary work. A visual assessment was made according to ASTM D5590-00 (2012). The results of antifungal coating resistance test showed that the best antifungal to remediate *A. niger* is potassium sorbate applied on thick wallpaper surfaces for plasterboard with growth percentages of about 47%. However, in the assessment of the inhibitory activity of *A. niger*, the results showed that concrete treated by zinc salicylate performed better than other antifungals used to inhibit *A. niger* with 50% on thick wallpaper covering. From this investigation, it was shown that the potassium sorbate antifungal was effective in decreasing the growth rate of the *A. niger*. However, the effectiveness of the results also depended on the construction building materials used. This study provides a reference in the inhibition of *A. niger* using different antifungals in various types of building materials. In addition, the effectiveness of antifungal compounds used in this study also exhibited enormous potential to be used as additives to prevent indoor fungal growth in Malaysia's construction industry.

ABSTRAK

Kualiti udara dalaman (IAQ) adalah penting untuk kesihatan dan keselesaan penghuni bangunan. Kepekatan kulat daripada udara, manusia, binatang dan lain-lain menjadi punca kepada sindrom yang dikenali sebagai bangunan sakit dan pencemaran udara dalaman. Dalam kajian ini, pertumbuhan kulat di bilik pensyarah, UTHM disiasat dan tiga jenis antikulat iaitu *potassium sorbate*, *calcium benzoate* dan *zinc salicylate* diaplikasikan. Kajian dijalankan untuk menghitung dan menilai pertumbuhan *Aspergillus niger* (*A. niger*) selepas menggabungkan antikulat pada empat kemasan dinding iaitu *acrylic paint*, *glycerol-based paint*, kertas dinding nipis dan kertas dinding tebal. Kemasan dinding yang telah digabungkan bersama antikulat diletakkan di atas permukaan bahan binaan yang berbeza iaitu *plasterboard*, konkrit dan kayu. 50ul *spore suspension* telah diletakkan di atas permukaan bahan binaan, diletakkan di dalam *petri-dish* yang mengandungi *malt extract agar*. Semua sample dieramkan pada kelembapan 90% suhu 32ºC, di mana data kelembapan dan suhu berpandukan optimum faktor pada kajian awalan. Penilaian secara visual adalah berdasarkan standard ASTM D5590-00 (2012). Keputusan ujian *coating resistance*, antikulat terbaik untuk mengurangkan pertumbuhan *A. niger* adalah *potassium sorbate* pada permukaan *plasterboard* yang disaluti kertas dinding tebal sebanyak 47%. Walau bagaimanapun, bagi peratus aktiviti perencatan *A. niger* terbantut pada permukaan konkrit disaluti kertas dinding tebal dengan 50% selepas dirawat dengan antikulat *zinc salicylate*. Antikulat *potassium sorbate* dan *zinc salicylate* dapat mengurangkan kadar pertumbuhan *A. niger* bergantung kepada jenis bahan binaan yang digunakan di dalam pembinaan pembangunan. Kajian ini dapat menyediakan rujukan dalam perencatan *A. niger* dengan menggunakan antikulat melalui ujian *coating resistance*. Selain itu penggunaan antikulat yang berkesan di dalam kajian ini mempunyai potensi untuk digunakan sebagai bahan tambahan untuk mencegah pertumbuhan kulat dalaman di dalam industri pembinaan di Malaysia.

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LIST OF SYMBOL AND ABBREVIATIONS

%	Percent
^{0}C	Degree Celsius
ASHRAE	American Society for Heating, Refrigerating and Air-Conditioning Engineers
ASTMD5590-00	American Society for Testing and Materials 5590-00
DOSH	Department of Occupational Safety and Health
EFSA	European Food Safety Authority
EPA	The United States Environmental Protection Agency
FKAAS	Faculty of Civil and Environmental Engineering
HVAC	Heating, Ventilation and Air Conditioning
ICOP-IAQ 2010	Industry Code of Practice on Indoor Air Quality 2010
IAQ	Indoor Air Quality
ISO 7730	International Standard Organization 7730
MEA	Malt Extracts Agar for Fungi
NIOSH	National Institute of Occupational Safety and Health
NMAM 0800	NIOSH Manual Analytical Method 0800
OSHA	Occupational Safety and Health Administration
RH	Relative Humidity
SBS	Sick Building Syndrome
TSA	Tryptocase Soy Agar
UTHM	Universiti Tun Hussein Onn Malaysia
VOC	Volatile Organic Compounds
WHO	World Health Organization

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Indoor air quality (IAQ) has become an important health topic since humans in general spend more than 80% of their time and activities in buildings. Humans may be exposed to various indoor air pollutants regardless of whether they are at their workplace or home (Wong *et al*., 2008). IAQ which is caused by any indoor toxins, for example chemical contaminants (carbon monoxide, formaldehyde, ozone, respiratory particulates and total volatile organic compounds (TVOC), biological contaminants (bacterial and fungal), ventilation performance indicator (carbon dioxide) and any mass can cause significant health effects. Therefore, further investigation is required in order to prevent bio-deterioration on different building materials.

1.2 Background of the study

Contaminated of indoor air is common in all buildings and can cause the spread of infectious diseases by airborne spread. Exposure to fungi in the building can cause several types of human health problems, especially itching, infections, allergies and certain toxic effects. It has been shown that toxigenic organisms are the reason for the unfavorable impact on wellbeing (Shelton *et al*., 2002) and contributes to a condition known as "sick building syndrome" (SBS). Therefore, it is imperative to discover an innovative approach to control or remediate the growth of indoor fungi.

There are five common contagious genera recognized in indoor air. They include *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria,* and *Aureabosidium* (Vacher *et al*., 2010; Bellotti *et al*., 2013; Verdier *et al*., 2014). The fungi species, *Cladosporium* and *Alternaria*, are mostly detected in outdoor air but have been detected in indoor air too. In addition, indoor contagious species such as, *Penicillium* and *Aspergillus* species can grow promptly on topsoil and rot litter (Hedayati, Mayahi, & Denning, 2010). Generally, fungi spread into the indoor environment through natural (open windows and doors) and mechanical ventilation systems (Gots, Layton, & Pirages, 2010; Laverge *et al*., 2011). The indoor environment is influenced by a few variables including temperature, dampness, water seepage into structures, and the amount of open air entering the building (D'Orazio *et al*., 2009; Deabes *et al*., 2011; Alghamdi *et al*., 2014).

In this study, the potential remediation of *Aspergillus niger* (*A. niger*) using a variation of antifungals has been explored. *A. niger* contains a toxin that can affect the health of those who work in the contaminated building. In addition, the effects of *A. niger* also contributes to sickness and allergic effects (Khan & Karuppayil, 2012; Flores *et al*., 2013; Verdier *et al*., 2014; Rakotonirainy, Vilmont, & Lavédrine, 2016).

The potential implications of indoor fungal growth to human health should not be underestimated; although the primary concern is the defacement of materials. The elimination and reduction of individual sources and their emissions are normally the effective method to control indoor air. Apart from that, the pollutants inside the building can be reduced when the amount of fresh air increases. In addition, humidity control helps to prevent moist air and decreases the growth of mould. However, humidity can be increased using a humidifier or decreased by opening the windows during dry weather (EPA, 2008).

The treatment of decay in buildings by synthetic antifungals has now been restricted due to their harmful effects on the environment, residual toxicity and carcinogenic nature (Khan & Karuppayil, 2012). Moreover, the constant use of chemicals has proven to induce resistance in a target organism. This concern has encouraged researchers to search for an efficient method to prevent and cure the harmful effects of microbes in a more eco-friendly manner (Sundis *et al*., 2012). Thus, there is a need to find a method of controlling the growth of fungi approach to address the IAQ problems.

Many researchers have investigated indoor air quality, but only a few of them focused on remediation of fungal growth. Thus, further study on remediation of indoor fungal growth using different antifungals on building material is required. Bellotti *et al*. (2013) recognized the use of bioactive compounds from the food industry which have the capacity to control mould development in indoor waterborne coatings. In their studies, it was found that the addition of antifungals such as zinc salicylate and zinc benzoate proved that these antifungals inhibited the species of *Chaetomium globosum* and *Alternaria alternate*. In addition, Vacher *et al*. (2010) reported that the Dibromo-3-Nitrilopropionamide was used as antifungal to treat *Penicillium* or *Aspergillus*, *Chaetomium*, *Ulocladium*, *Stachybotrys* and *Cladosporium* on biodegradable materials. The antifungal nano zinc oxide and anatase titanium dioxide were found as the best photocatalytic agent and broadest spectrum of antimicrobial agents for interior paints to remediate *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* and *Penicillium chrysogenum* (Hochmannova & Vytrasova, 2010). Another study by Stanojevic *et al*. (2009) confirmed the potential of sodium benzoate, sodium nitrite, and potassium sorbate to reduce *Bacillus subtilis*, *Bacillus mycoides*, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus flavus, Fusarium oxysporum, Candida albicans, Trichoderma harsianum* and *Penicillium italicum* and *Escherichia coli*.

Smilanick *et al*. (2008) highlighted the use of potassium sorbate, imazalil (imz), thiabendazole (tbz), pyrimethanil, and fludioxonil and found that the potassium sorbate was succeessful in remediating *Penicillium digitatum.* According to Clausen & Yang, (2007), potassium sorbate was found to inhibit the growth of about 5% for several types of fungus such as *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma viride* from outdoor sources. These studies showed that the antifungal compounds from various kinds of species have some potent antifungal ability.

However, the application of these antifungal in remediation of indoor microbial and fungal contamination has not been given full attention, especially in Malaysia. This is the motivation behind the present study. To best of the author's knowledge, no report was found on the evaluation of *A. niger* remediation using antifungals (potassium sorbate, calcium benzoate and zinc salicylate) applied on building materials. These antifungals were selected based on a previous study that showed that the antifungals which were applicable in controlling indoor fungi were mostly used in the food industry. However in this study, these antifungals is explored

explored for their use as antimicrobial additives for antimicrobial coatings in the construction industry, with concern placed on the safety of chemical used. The assessment of indoor air quality should be carried out and must take into consideration the safety of indoor occupants (DOSH, 2010). However, only several studies on indoor fungal remediation have been conducted at new buildings in this country. Rahman *et al*. (2012) conducted their investigation on the controlled growth of fungus in university's clinic in Malaysia but not in faculty buildings in Malaysia. Another study carried out in a secondary school building, found that fungal DNA and cat allergen were common in the Malaysian school (Halstensen, 2008).

Other researches to date tended to focus on others various species (Belloti *et al,* 2013; Duangnapa, 2012; Deabes *et al,* 2012) rather than that *A. niger* specifically. Therefore, the aim of the present study is to remediate the *A. niger* especially for common interior finishings of a building using several types of antifungals which include potassium sorbate, calcium benzoate and zinc salicylate that were previously used in food preservatives. It is hoped that this study would produce a new application to remediate the *A. niger* growth, after incorporating different wall finishings with antimicrobials coating. This was motivation for the present study.

1.3 Problem statement

Nowadays, issues concerning air quality with regard to indoor health environment are nothing new as they have been studied since 1850 during the hygienic revolution, and then followed by outdoor environmental issues (Spengler *et al*., 2002). Similarly in Malaysia, indoor air quality has become prevalent recently to be explored. Although some buildings meet the recommended overall standard, there have been times that occupants would still complain about various parameters such as wall finishing and materials which are still not up to pay with satisfying consumer needs. Thus, it is essential for the building to have a good indoor air quality (IAQ) as it affects the productivity and health of the occupants in the building. In fact, sustainable development results are not just in resource conservation only, but also in increasing productivity and occupant well-being (Syazwan *et al*., 2009). More research needs to be conducted on issues regarding IAQ, especially on buildings in Malaysia with regarding to the established standards that should be developed for the benefit to the occupants. Therefore, any IAQ issues related to residential, commercial, office and

institutional buildings where contamination has occurred by a variety of toxic or hazardous substance, as well as pollutants of biological origin requires serious solutions. This is due to the fact that microbial pollutants can cause the infection to the occupants in the building. For instance, fungus produces toxic mycotoxins, endotoxins, glucans and others in which are very harmful to human health (Verdier *et al*., 2014).

Previous studies carried out in temperate region such as Japan's climate, with different periods of high temperatures and relative humidity were reasonable to the development of fungi (Nazar *et al*., 2013). Similarly, Shelton *et al*. (2002) found that the occurrence of indoor fungus in the indoor environment was prevalent during the fall and summer seasons in the United States. According to Frankel *et al*. (2014), high relative humidity and temperature are among the factors that greatly contribute to the higher occurrence of indoor fungus. Additionally, others factor may influence indoor fungal growth, like the weather itself. For example, in Malaysia the weather is hot and humid during day and this leads to higher fungal growth at night. Furthermore, many of studies have shown that there is a relationship between indoor fungus and humidity to the health effect. This has also been shown to play a role in the production of mycotoxins (Vacher *et al*., 2010). The temperature averages between 25ºC and 35ºC indicating a high indoor temperature, which is normally associated with local climate in Malaysia. However, this temperature range for the climate in Malaysia is in good agreement with DOSH (2010) where the best indoor temperature is supposed to be between 23ºC and 26ºC.

To date, there is still a lack in studies in Malaysia regarding the remediation of indoor fungus using antifungals in the construction industry. All the building materials currently used in construction, easily exposure to of mould growth especially in extreme moisture condition. The normal cleaning approaches however cannot provide an effective and sustainable solution to this mould problem. The continued replacement of damaged construction building materials with the new ones is neither cost-effective nor environmental friendly. This approach is not effective in Malaysia because of the high humidity throughout the year. Thus, there is a significantly need to search for sustainable, long term, low cost and environmental friendly approaches to address the IAQ problems. The issue of fungus in buildings is not new in Malaysia as it has been reported in the media and has occurred in hospitals, schools and various government buildings.

Recently, the UTHM authorities have received complaints of indoor fungal growth in the offices of academic buildings. As a result, UTHM's management has launched a research to find a lasting solution to this issue. The management is concerned that the fungus will affect the health of the lecturers and students. Therefore, the problem being addressed in this study is new as the application of the three selected antifungals (potassium sorbate, calcium benzoate and zinc salicylate) that are able to inhibit the growth of fungus in building materials (plasterboard, concrete and wood) are covered by different types of paint and wallpapers in Malaysian climate.

1.4 Research questions

This study was carried out to investigate the following research questions:

1) How the relative humidity and temperature levels influence the existence and growth of indoor fungal contamination?
2) What is the growth trend of *A. niger* when incorporated with optimum humidity of tropical climate?
3) How efficient the potassium sorbate, zinc salicylate and calcium benzoate antifungals used for coating bio-resistance test with different wall finishing can remediate or prevent *A. niger* on the substrates?

1.5 Objective of study

This study embarks on the following objectives:

1) To evaluate contamination of air in a room according to the Industry Code of Practice on Indoor Air Quality, 2010.
2) To optimize the specific growth rate of *A. niger* at different levels of temperature and relative humidity.
3) To develop a new application of coating resistance using antifungals in remediation specifically of *A. niger* on construction building materials covered with different wall finishing.
4) To compare the inhibition of *A. niger* growth after incorporation on three variations of treated wall finishing substrates.

1.6 Scope of study

1) This study used indoor fungal, *Aspergillus niger* that is isolated from a building. Selected antifungals consisted of potassium sorbate, calcium benzoate, and zinc salicylate, which were used to prevent indoor fungal growth from the air in the affected building.
2) The methods applied to collect indoor fungal are the single stage impactor (biosampler) in accordance with prerequisite of National Institute of Occupational Safety and Health (NIOSH) method (NIOSH Manual Analytical Method NMAM 0800).
3) Physical and chemical IAQ parameters were analyzed using guidelines of Industry Code of Practice of Indoor Air Quality in Malaysia (ICOP-IAQ) – DOSH, 2010.
4) The selected specific indoor fungus was inoculated at the concentration of 0.3 X 10^6 on substrates such as plasterboard, wood, and concrete.
5) In the coating resistance test, four wall finishing product were used on the plasterboard, concrete and wood surface which were acrylic paint, glycerol-based paint, thin wallpaper (80 g/m^2) and thick wall paper (240 g/m^2); and the indoor fungal growth was evaluated according to the ASTM D5590-00 (2012) standard scale.

1.7 Significance of study

The findings of this study contribute to the knowledge and understanding of the indoor fungal treatment using selected antifungals. This study is significant because:

1) The selections of the antifungal applied in this research have not been studied yet for indoor fungal treatment to remediate the *A. niger* that was isolated from indoor building especially in institutional buildings.
2) This study combines the function bio-coating resistance test using different wall finishing on different building material.
3) A new study of antifungal in combination with different wall finishing to remediate indoor fungal growth.
4) The selection of antifungal as antimicrobial additives for indoor fungal growth remediation can minimize the cost of renovation, demolition,

and reconstruction of sick building.

5) Provide an alternative prevention to avoid any hazardous to occupant and materials damage in building.

1.8 Thesis overview

This dissertation consists of five chapters. The chapter 1 describes the background of the study, problem statement, objectives, scope of study, and significance of study. Meanwhile chapter 2 explains a critical review of the research about indoor air quality, source of indoor air quality, classification of indoor air contaminations, health effects due to poor IAQ and the method to the remediation of poor IAQ. Chapter 3 provides in details the method of development used in this study including indoor fungal preparation, optimization of indoor fungal growth, the bio-coating resistance test and inhibition performance of *A. niger*. Then, chapter 4 is the result and discussion on the optimum growth of indoor fungus via response surface methodology and analysis on the growth percentages of *A. niger* treatment. Finally, chapter 5 is the conclusion for the overall objectives setup and further works to be recommended in the future.

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